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Comparison of DNA Adducts from Exposure to Complex Mixtures in Various Human Tissues and Experimental Systems

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DNA adducts derived from complex mixtures of polycyclic aromatic compounds emitted from tobacco smoke are compared to industrial pollution sources (e.g., coke ovens and aluminum smelters), smoky coal burning, and urban air pollution. Exposures to coke oven emissions and smoky coal, both potent rodent skin tumor initiators and lung carcinogens in humans, result in high levels of DNA adducts compared to tobacco smoke in the *in vitro* calf thymus DNA model system, in cultured lymphocytes, and in the mouse skin assay. Using tobacco smoke as a model in human studies, we have compared relative DNA adduct levels detected in blood lymphocytes, placental tissue, bronchoalveolar lung lavage cells, sperm, and autopsy tissues of smokers and nonsmokers. Adduct levels in DNA isolated from smokers were highest in human heart and lung tissue with smaller but detectable differences in placental tissue and lung lavage cells. Comparison of the DNA adduct levels resulting from human exposure to different complex mixtures shows that emissions from coke ovens, aluminum smelters, and smoky coal result in higher DNA adduct levels than tobacco smoke exposure. These studies suggest that humans exposed to complex combustion mixtures will have higher DNA adduct levels in target cells (e.g., lung) as compared to nontarget cells (e.g., lymphocytes) and that the adduct levels will be dependent on the genotoxic and DNA adduct-forming potency of the mixture.

Introduction

Biomarkers of human exposure to complex mixtures have only recently been developed. In the past, analyses of tracer compounds (e.g., benzo[*a*]pyrene and nicotine) have typically been used as surrogates for the entire mixture. The development of ^{32}P-postlabeling methods (*1,2*) for detecting DNA adducts covalently bound to DNA has had a dramatic impact in facilitating the measurement of exposure to complex mixtures at the DNA level (*3*). This method allows hundreds of bulky aromatic DNA adducts to be detected simultaneously at extremely low detection limits without structural knowledge of the specific adducts being detected.

This paper presents both human and experimental studies of DNA adducts formed after *in vitro* and *in vivo* exposures to specific complex emission sources using ^{32}P-postlabeling methods. The human studies rely primarily on tobacco smoke exposure. We have compared the level of DNA adducts in white blood cells (WBC), lymphocytes, placental tissue, lavaged lung cells, sperm, and autopsy tissues of smokers and nonsmokers. Tobacco smoke is contrasted to other environmental and occupational exposures in human and experimental systems.

Materials and Methods

Experimental Exposures to Complex Mixtures

The collection, sample handling, and chemical characterization of the complex mixtures used in this study are described in detail elsewhere and are summarized here (see specific citations below). In general, the respirable combustion particle emissions were collected after dilution onto filters, with the exception of several condensates, which are specified below. The organics adsorbed onto particle emissions were extracted using dichloromethane (DCM) and stored at −80°C either in solvent or dried under nitrogen.

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The tobacco smoke samples used in these studies are mainstream and sidestream cigarette smoke condensate collected by standard procedures using Kentucky 2R1 Reference Cigarettes (*4–6*). The coke oven emissions were collected from the separator between the gas collector main and primary coolers within the coke oven battery at a coke oven in Gadsden, Alabama (*4–6*). The diesel vehicle emissions were collected by dilution tunnel sampling of a Volkswagen Rabbit vehicle operated on a chassis dynamometer using simulated driving patterns (*4–6*). The smoky coal emissions were collected by high-volume particle samplers operated indoors in Xuan Wei, China, during indoor cooking using smoky coal in homes without chimneys (*7*). Aluminum smelter emissions were collected using a dilution and bag house sampling device connected to the roof vents of an active aluminum smelter using the Soderberg process in Tacoma, Washington. The roof vent was located in the roof of the building housing the smelter pots.

Air particles were collected in the winter of 1986–1987 using high-volume air samplers with 2.5-μm impactors and Teflon-impregnated glass fiber filters. The samplers were located in Boise, Idaho, at a residential site where the primary emissions were woodsmoke and at a highway intersection where vehicles (gasoline cars and diesel trucks) as well as some woodsmoke were the primary emission sources. The sample used here was a composite sample containing both woodsmoke and mobile source emissions (*8*).

In Vitro Calf Thymus DNA Model System

The *in vitro* assay is described in detail by Gallagher et al. (*9*). In this assay, calf thymus DNA (1 mg/mL) was incubated for 1.5 hr at 37 °C with the complex mixtures in the presence of rat liver S9 (0.5 mg/mL), pH 7.5, in a final incubation volume of 2 mL.

Cultured Human Lymphocyte Assay

Whole blood was collected in heparinized sterile tubes from six individuals, and lymphocytes were isolated on Ficoll Paque (Pharmacia Chemical Co., Piscataway, NJ) (*10*). The isolated lymphocytes were cultured as previously described (*11*). The complex mixtures (1 μg/mL) and benzo[*a*]pyrene (BaP) (1 μM) were added to the culture medium for 18 hr at 37 °C in supplemented media devoid of mitogen.

Mouse Skin Assay

The mouse skin exposure and DNA adduct analysis were conducted as previously described (*12*) for the coke oven, diesel, and smoky coal emissions, with the exception of the studies of aluminum smelter and urban air samples. These studies were conducted in Sencar mice in conjunction with tumor initiation assays. The data reported here are for a single 5-mg dose. Complete dose–response studies are in progress.

Human Lymphocytes

The human lymphocytes used in this study were collected as described above and isolated as previously described (*10*) except using Histopaque 1077 from Sigma Diagnostics (St. Louis, MO). Blood was collected from healthy males 34–57 years old, smokers and nonsmokers, who lived in the town and surrounding countryside of Ostrava, Czechoslovakia. This population also included a group of coke oven workers.

Human Sperm

Semen specimens were provided by healthy white males 18–35 years old in Chapel Hill, North Carolina. The exposure groups included 12 heavy smokers (20 or more cigarettes per day for at least 1 year), and 12 nonsmokers (less than 100 cigarettes in their lifetime) (*13*). Human sperm cells were washed in phosphate buffered saline at 4°C and stored in 150 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 7.4 at −70°C.

Human Placental Tissue

The human placenta samples were collected from healthy pregnant volunteers on delivery at the obstetrical service of the University of North Carolina Hospital. Women in the exposure groups were classified based on their smoking history. Placentas were frozen at −80 °C shortly after delivery (*14*). Placentas from normal term pregnancies were also collected from women living in China who were heavily exposed to either smoky coal in open fires used for heating and cooking or from women who used natural gas (*7*).

Human Lung Lavage Cells

Human lung cells, consisting primarily of macrophages, were collected by bronchoalveolar lavage (BAL) from healthy smokers and nonsmokers. The detailed procedures are essentially as described by Koren et al. (*15*). In brief, the volunteers for this study included only healthy males between 18 and 35 years of age. A total of 300 mL of normal saline was instilled in five instillations. The recovered cells were >85% viable as measured by trypan blue dye exclusion. The BAL samples were placed on ice and then immediately centrifuged at 1100 rpm for 10 min at 4°C and frozen at −70°C. In a separate study, using similar procedures, BAL cells were from cancer patients in Xuan Wei, China, exposed to smoky coal (*16*). The controls were from Kunming, China, where either electricity or natural gas is used in the home for cooking rather than smoky coal (*17*).

Human Autopsy Tissues

Human lung, heart, and other tissues were collected at the University of North Carolina Memorial Hospital. The tissues were from three exposure groups: smokers, former smokers, and nonsmokers. The tissues were stored frozen at −80°C.

DNA Isolation

DNA from the calf thymus system and rodent tissues was isolated as described by Gupta (*18*). DNA from human lymphocytes treated *in vitro* was isolated as described by Maniatis et al. (*19*). In studies where the DNA was limited, as in the case of the human studies of lymphocytes and BAL cells, we modified the procedure of Gupta et al. (*18*) by reducing the total volumes of the lysing and organic extracts and treated sequentially with a mixture of RNAse (A and T_1) and proteinase K. In these cases,

the number of extractions were limited to one chloroform/isoamyl alcohol (Sevag)/phenol (1:1) and one Sevag only. Sperm and autopsy tissue DNA was isolated using the Applied Biosystems (Foster City, CA) Model 340 A Nucleic Acid Extractor using the manufacturers reagents. β-Mercaptoethanol was added to the sperm cell lysate to increase the yield of DNA.

^{32}P-Postlabeling Analysis

^{32}P-Postlabeling analysis was conducted using previously reported modifications (*20*) of the methods of Gupta [(*21*) referred to in this study as the butanol extraction procedure] and Reddy and Randerath [(*22*); referred to as the nuclease P1 procedure]. For samples in which both the butanol extraction and nuclease P1 version of the ^{32}P-postlabeling methods were used, the digest was divided into two aliquots. DNA adducts were enriched from one aliquot by the butanol extraction version of the ^{32}P-postlabeling assay and the other by the nuclease P1 treatment. With either version 5.0 or 10 μg of DNA was incubated with approximately 50 μCi [γ-^{32}P]ATP (Amersham, Arlington Heights, IL; 3000 Ci/mmole) and 3.5 units T_4 polynucleotide kinase for approximately 30 min. DNA was spotted onto polyethyleneimine (PEI) TLC plates. The adducts were resolved using previously described solvent systems (*20*). In two of the studies of tobacco smoke, human sperm and lymphocytes, we report the use of the magnet contact transfer method. In these cases, after postlabeling, the DNA digests were transferred to fresh PEI plates using the magnet contact transfer method as described by Lu et al. (*23*).

To measure the total number of nucleotides, an aliquot (0.5 μg of mononucleotides) was diluted approximately 200-fold and 2.5 μL labeled with an equivalent amount of radiolabeled mix. A further 80-fold dilution was made and 5 μL spotted on a PEI-cellulose plate previously pretreated with 100 mM ammonium formate, pH 3.5, and developed in one dimension with 4.5 M ammonium formate, pH 3.5.

Intensifying-screen-enhanced autoradiography at −80 °C was used to detect the presence of radiolabeled adducts on the TLC plates. The DNA adducts that migrated along a diagonal radioactive zone (DRZ) were carefully scraped with the aid of a template, which outlined the boundary of radioactivity. Ethanol (5.0 mL of 95%) was added to each vial and Cerenkov counted (*24*) using a scintillation counter. Relative adduct levels were determined from the counts per minute detected for the excised areas divided by the counts per minute determined for the total nucleotides after correcting for dilution factors, background radioactivity, and micrograms of DNA spotted.

Results and Discussion

DNA adducts have been detected and quantitated using ^{32}P-postlabeling methods after *in vitro* and *in vivo* exposures to specific complex emission sources in human and experimental studies. The human studies comparing target and nontarget cells and tissues used tobacco smoke exposure to compare the level of DNA adducts in blood lymphocytes, placental tissue, lavaged lung cells, sperm, and autopsy tissues of smokers and nonsmokers. Tobacco smoke is contrasted to other environmental and occupational exposures in humans and experimental systems. The Results and Discussion section is organized to address the following questions: *a*) Using experimental models, can we predict the nature of DNA adducts that may be formed in humans from various complex mixtures? *b*) Which human cells and tissues may provide the most useful information in human biomarker studies of DNA adducts resulting from exposure to complex environmental mixtures?

In Vitro DNA Model System

The calf thymus DNA experimental system is the most sensitive assay for detecting and characterizing chromatographic properties of complex mixture-derived DNA adducts formed under specific activation protocols. The activation systems discussed here include xanthine oxidase, which reduces nitroarene (e.g., nitro-polyaromatic hydrocarbon) and related compounds to DNA adducts that are nuclease P1 sensitive [e.g., *N*-(deoxyguanosin-8-yl)-1-aminopyrene], and the Aroclor-induced mammalian microsomal system (S9) used in the Salmonella mutagenicity assay (*9*). Comparison of a series of the complex mixtures listed above confirms that with calf thymus DNA in the presence of an S9 activation system, most of these mixtures form DNA adducts that migrate along a DRZ as illustrated in Figures 1 and 2. DNA adduct profiles for various mixtures are distinctly different and depend on the activation system used. For example, greater than 60% of the diesel-derived adducts formed after xanthine oxidase treatment are not detectable after nuclease P1 treatment (*20*) compared to DNA adducts derived from S9-mediated microsomal activation (*9*).

The relative potency of these different mixtures as measured by the total adduct level in the DRZ per milligram organic matter per milliliter in calf thymus DNA after S9 microsomal activation is shown in Table 1. Cigarette smoke is the weakest mixture with respect to DNA adduct formation and coke oven emissions the most potent, with a more than 20-fold difference between these two mixtures in DNA adduct-forming efficiency.

Cultured Human Lymphocyte Assay

DNA adducts have been detected in human lymphocytes treated *in vitro* with smoky coal, diesel, and coke oven extracts (*25*). To date, we have not detected DNA adducts in human lymphocytes treated *in vitro* with mainstream (MS-CSC) or sidestream (SS-CSC) cigarette smoke condensate as shown in Figure 1, even though these constituents are activated to DNA reactive intermediates via rat liver S9-mediated metabolism in our *in vitro* calf thymus experimental system as seen in Figure 1.

The individual variation between human blood donors results in substantial differences in the DNA adduct levels between individuals for the various complex mixture-derived DNA adducts. We have not, therefore, included the lymphocyte results in Table 1. Nevertheless, the relative adduct levels observed in several individuals for the diesel-derived DNA adducts are higher than those for the coke oven or smoky coal. This is in contrast to the calf thymus DNA and mouse skin assay systems shown in Table 1, where coke oven emissions are clearly more potent than diesel emissions. The differences we have observed in the nature and level of DNA adducts between the *in vitro* DNA and lymphocyte systems suggest that major differences exist in the metabolic activation capabilities of lymphocytes compared to the Aroclor-induced rat liver S9. There are, however, obviously other differences in these two *in vitro* assays. These differences

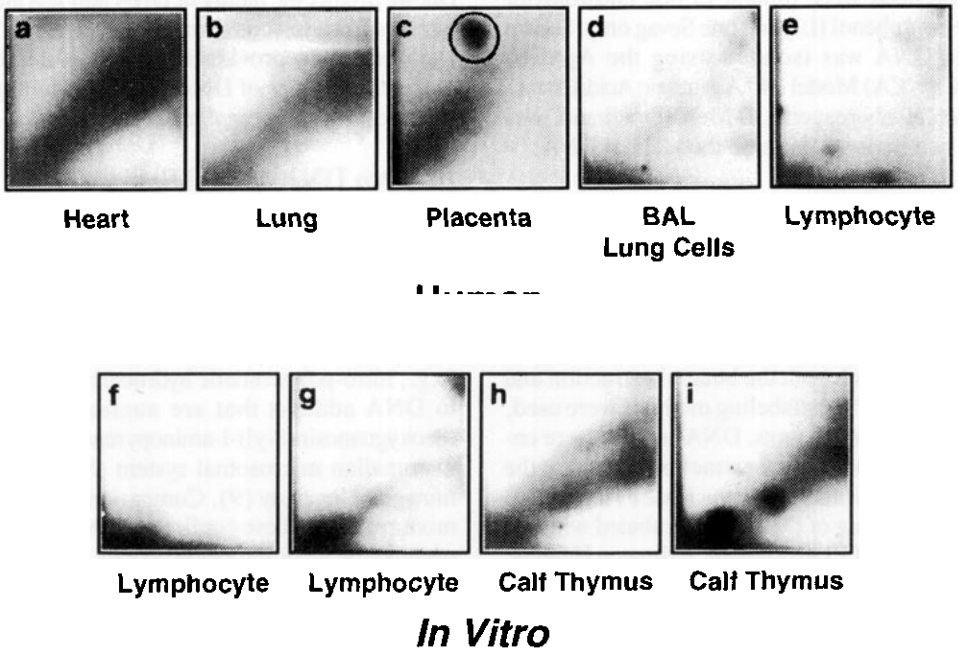

FIGURE 1. Autoradiograms of DNA adducts detected in human tissues/cells *(a–e)* isolated from smokers: (*a*) heart, (*b*) lung, (*c*) placenta, (*d*) bronchoalveolar cells (BAL), and (*e*) blood lymphocytes. DNA adduct patterns were similar for heart, lung, and placental DNA; however, one additional DNA adduct (*c*, circled) was identified in placental tissue. (*f–i*) DNA adducts resulting from *in vitro* exposures. Human lymphocytes were treated with mainstream (MS-CSC) and sidestream (SS-CSC) smoke (*f,g*, respectively). Calf thymus DNA adducts resulting from rat liver S9-mediated metabolism of MS-CSC (*h*) and SS-CSC (*i*).

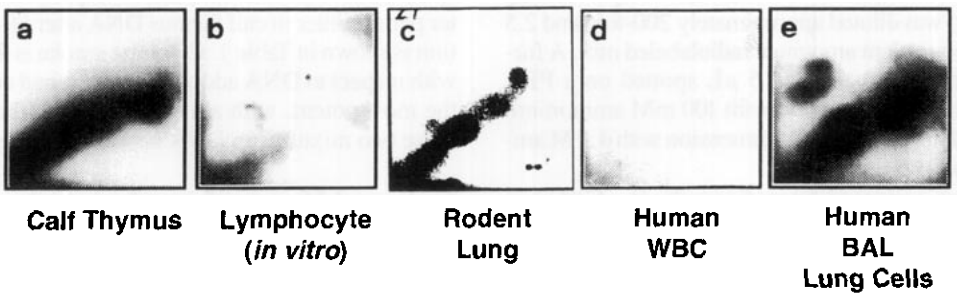

FIGURE 2. Autoradiograms of DNA adducts after *in vitro* (*a,b*) and *in vivo* rodent (*c*) and human (*d,e*) exposures to smoky coal. (*a*) Calf thymus DNA adducts resulting from S9-mediated metabolism of smoky coal extract; (*b*) human lymphocyte DNA after *in vitro* treatment with 1 μg/mL smoky coal; (*c*) rodent lung DNA after topical application of smoky coal organic extracts; (*d*) human white blood cell DNA from an individual exposed to smoky coal; (*e*) human bronchoalveolar cell DNA isolated from individuals exposed to smoky coal.

are related to the nature of the chemical exposure to DNA (e.g., direct contact with calf thymus DNA versus uptake and transport into lymphocyte chromosomal DNA).

Mouse Skin Tumor Initiation Assay

The mouse skin tumor initiation model has been widely used to evaluate the tumor initiating activity of polycyclic aromatic compounds (PAC) and complex mixtures containing PAC (*26*). The relative tumor-initiating potency of emissions from coke ovens, roofing (coal) tar, and cigarette smoke has been shown to highly correlate with the human lung cancer potency of these same three mixtures (Table 1)(*5*). Therefore, we have compared tumorigenicity in the mouse skin model with DNA adduct formation with similar exposures (*12*).

DNA adducts were detected in the skin and lung DNA 24 hr after skin application of organic extracts of coke oven, aluminum smelter, diesel, smoky coal, and urban air particles (Fig. 3). Similiar studies have been conducted by other investigators using MS-CSC (*27*), coal tar, and related mixtures (*28,29*). Although relative adduct levels were generally higher in the skin, the autoradiograms of DNA adducts from the lung more clearly show the presence of dicrete DNA adducts (Fig. 3). Characteristic differences in DNA adduct chromatographic patterns for each of these mixtures are shown in Figure 3. One major DNA adduct detected in lung DNA for all complex mixtures migrated with a major BaP-derived DNA adduct detected in skin DNA after topical application with BaP. Based on the relative

Table 1. Potency of complex mixtures in forming DNA adducts in experimental systems compared to tumor and cancer potency.

Complex mixture	DNA adducts *in vitro*[a]	DNA adducts in mouse skin/ mouse lung[b]	Tumor potency[c]	Human lung cancer risk
Cigarette smoke	104	0.5/0.7	0.002	0.02×10^{-4}[d]
Coke oven	2378	7.2/5.2	2.1	9.3×10^{-4}[d]
Smoky coal	772	3.8/1.6	2.7	—
Diesel	465	0.6/0.4	0.24	0.7×10^{-4}[e]
Aluminum smelter		3.7/3.6	0.76	
Urban air		1.6/1.1	0.21	

[a]Relative adduct levels × 10^8/mg mixture/mL for the *in vitro* calf thymus DNA system with S9 activation and nuclease P1 version of the postlabeling assay.

[b]Skin/lung relative adduct levels × 10^8 mg mixture applied to the skin at the 20-mg dose, except for the cigarette smoke, which is from Randerath et al. (*27*) at the 0.18 mg dose; aluminum smelter and urban air at 5-mg dose. The relative potency of the coke oven, smoky coal, and diesel is an underestimate due to the relatively high dose used.

[c]Papillomas/mouse/milligram in Sencar mice (*5*).

[d]Lifetime excess lung cancer risk/microgram organic matter/cubic meter estimated from human epidemiological data (*5*).

[e]Estimated from a lifetime rodent inhalation study (*26*).

concentration of BaP in these mixtures, however, it is unlikely that this adduct is derived from BaP alone (*12*). This is especially true for the diesel emissions, which contain a higher proportion of mutagenic, polar, nitrated PAC (*30*). The DNA adducts as detected in mouse lung are chromatographically similar to calf thymus DNA adducts for diesel-derived DNA adducts (*9*) and smoky coal-derived DNA adducts, as shown in Figures 2*a* and 3*d*.

We have consistently found that the DNA adduct-forming efficiency for these mixtures in mouse skin is higher at lower doses (*12*). To estimate the relative potency of DNA adduct formation of these mixtures, the initial linear slope of the dose response for DNA adduct formation should be measured. Unfortunately, initial linear slopes are not yet available for all the mixtures described here; however, the available data for skin and lung DNA adduct-forming potency (efficieny) are shown in Table 1 with dosimetry limitations stated in footnote to Table 1. In spite of these limitations, the relative ranking of the mixtures with respect to DNA adduct formation *in vitro* and in the mouse skin is similar to each other and to the relative ranking of tumor potency and human cancer risk estimates. Tobacco smoke is the weakest complex mixture we have have evaluated with respect to formation of DNA adducts *in vitro*, and based on studies of tobacco smoke by Randerath et al. (*28*), tobacco smoke ranks as the weakest mixture with respect to DNA adduct formation in mouse skin and lung after skin application, as shown in Table 1.

Human Cells and Tissues

Humans exposed to tobacco smoke provide the best opportunity to compare DNA adduct formation in various cells and tissues,

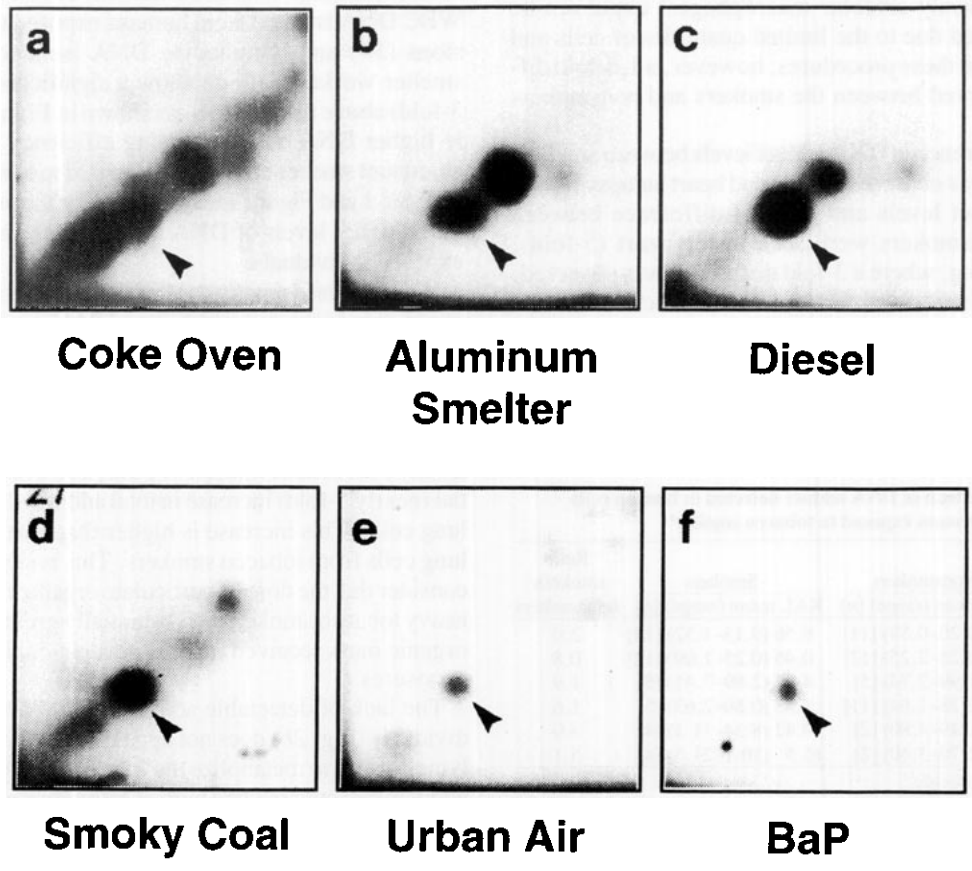

FIGURE 3. Autoradiograms of DNA adducts detected in rodent lung DNA after topical application of (*a*) coke oven, (*b*) aluminum smelter, (*c*) diesel, (*d*) smoky coal, and (*e*) urban air particle extract. In all cases, one major DNA adduct (arrowhead) was detected for all complex mixture-modified DNA that co-migrated with a BaP–DNA adduct detected in skin DNA after topical application with BaP (*f*).

including both target (e.g., lung) and nontarget tissue (e.g., lymphocytes). Comparison of the relative adduct levels detected in nonsmokers versus smokers for a series of different human studies are shown in Table 2. Significant differences in DNA adduct levels between smokers and nonsmokers have not consistently been observed in studies of white blood cells or blood lymphocytes (*11,31–34*). In those postlabeling studies reporting a difference in DNA adduct levels, the investigators isolated lymphocytes (*35,36*) and the effect of smoking was not greater than 2.5-fold. In the relatively small study we report here, we were able to detect a 2-fold difference in lymphocyte DNA adduct levels between smokers and nonsmokers when the adducted nucleotides were contact transferred via a magnet to fresh PEI plates, as shown in Table 2. Whether minimizing background problems (commonly associated with long-term film exposures) will assist in providing clearer differences with respect to DNA adduct levels in smokers and nonsmokers needs to be investigated further using a larger database. Savela and Hemminki (*35*) suggest that isolation of the lymphocytes from the WBC population may also be required to detect smoking-related adducts.

DNA from sperm cells did not show a difference in DNA adduct levels between smokers and nonsmokers using the magnet contact transfer method. Placental tissue used in these studies consistently showed a 2-fold difference between smokers and nonsmokers on repeated analysis. The lung lavage cells (determined to be primarily alveolar macrophages) could not be repeatedly analyzed due to the limited quantities of cells and DNA isolated from these procedures; however, a 1.6-fold difference was observed between the smokers and nonsmokers (Table 2).

The largest difference in DNA adduct levels between smokers and nonsmokers was observed in lung and heart autopsy tissue. The highest adduct levels and greatest difference between smokers and nonsmokers were seen in the heart (5-fold), followed by the lung, where a 3-fold difference was observed. Former smokers also showed elevated DNA adduct levels in the heart (3-fold) and lung (2-fold) (data not shown). Figure 4 illustrates the DNA adduct patterns detected in heart, lung, and liver DNA isolated from smokers. The DNA adduct patterns in heart were similar to lung. A secondary DRZ more polar than the primary DRZ was detected in heart and lung DNA in the nuclease P1-treated samples (Fig. 4). This secondary zone was not apparent in the butanol-treated heart and lung DNA samples and may be indicative of the poor recovery of polar adducts in the butanol extraction procedure.

Table 2. Comparison of DNA adduct detected in human cells and tissues exposed to tobacco smoke.[a]

Human cell tissue	Nonsmokers RAL mean (range) [*n*]	Smokers RAL mean (range) [*n*]	Ratio smokers/ nonsmokers
Lymphocytes[b]	0.28 (0.20–0.59) [11]	0.56 (0.13–1.32) [11]	2.0
Sperm[c]	0.60 (0.25–2.25) [12]	0.45 (0.25–1.09) [12]	0.8
Placenta	2.34 (1.99–2.74) [5]	4.42 (2.89–7.41) [5]	1.9
Lung lavage	0.97 (0.38–1.64) [11]	1.53 (0.80–2.63) [5]	1.6
Lung	3.15 (1.49–4.80) [2]	9.42 (8.34–11.1) [4]	3.0
Heart	3.01 (2.70–3.40) [2]	15.5 (10.1–21.5) [4]	5.1

RAL, relative adduct levels.

[a]DNA adduct levels reported in RAL $\times 10^8$ with the mean and range shown. The number of individuals in each study is shown in brackets. The data presented here are derived using the P1 nuclease version of the ^{32}P-postlabeling assay.

[b]Data shown are from postlabeling studies using the magnet contact transfer method (*23*). Without contact transfer, the mean and range for the nonsmokers and smokers were 0.67 (0.33–1.34) and 0.68 (0.21–1.44), respectively.

[c]Data shown are from the magnet contact transfer method. No difference was detected in the absence of contact transfer.

One major DNA adduct in both smoker and nonsmoker heart, lung, and liver DNA was detected only using the butanol extraction method as shown in Figure 4, suggesting that this adduct is nuclease P1 sensitive. Because many nitroarenes and *N*-substituted arylamine DNA adducts are dephosphorylated by nuclease P1 (*20,37*), these apparently ubiquitous adducts may be derived from nitroarenes or arlyamines either present in the environment (e.g., ambient air) or diet, or they may represent an endogenously formed adduct. Whether the nuclease P1-sensitive adducts that we detect in heart, lung, and liver are present in other human tissues is currently under investigation.

A comparison of the DNA adducts in humans exposed to tobacco smoke compared to controls is shown in Figure 5. A 1.45- to 2.4-fold increase in adducts related to the tobacco smoke exposure was observed for the three studies in which DNA was analyzed from blood lymphocytes. The largest increase in DNA adducts resulting from tobacco smoke exposure was observed in heart DNA.

Based on the evidence from experimental *in vitro* and *in vivo* assays, we can hypothesize that human exposure to emissions from coke ovens, aluminum smelters, and smoky coal would result in formation of DNA adducts. Previous studies in which WBC DNA isolated from humans exposed to coke oven emissions (*34*) and lymphocyte DNA isolated from aluminum smelter workers (*36*) do show a significant increase (2.4- to 3-fold) above the controls, as shown in Figure 5. The relatively higher DNA adduct-forming efficiency of coke oven and aluminum smelter emissions compared to tobacco smoke shown in Table 1 and Figure 3 may account for the presence of substantially higher levels of DNA adducts in the blood cell DNA of exposed individuals.

We have used smoky coal emissions as a model for humans exposed to high concentrations of PACs in the air. Figure 2 illustrates the DNA adduct profiles detected in the *in vitro* experimental systems and *in vivo* rodent compared to the human samples. We did not detect DNA adducts in WBC or in the placental tissue DNA of nonsmoking women highly exposed to smoky coal in their homes. We did, however, detect a substantial (nearly 5-fold) increase in total adducts detected in the BAL lung cells. This increase is higher than that observed in BAL lung cells from tobacco smokers. This is significant when you consider that the dose of particulate organic matter (e.g., tar) to heavy tobacco smokers is substantially greater than the dose of organic mass received in most occupational or environmental exposures.

The lack of detectable adducts in the WBC of exposed individuals (Fig. *2b*) does not appear to be due to the inability of lymphocytes to metabolize the PAC in smoky coal based on the adducts detected *in vtiro* with cultured lymphocytes (Fig. *2a*). It is possible, however, that isolation of lymphocytes from the mixture WBC population would increase our ability to detect DNA adducts as reported by Savela and Hemminki (*35*) for tobacco-smoke-related adducts.

We postulate that a relatively greater effect on DNA adduct formation by smoky coal and the other emissions with high con-

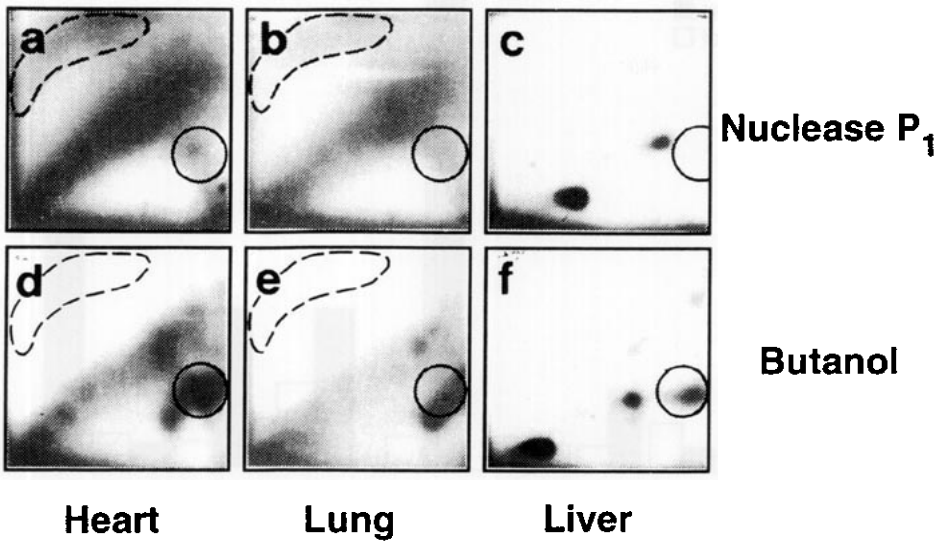

FIGURE 4. DNA adducts detected in human (*a,d*) heart, (*b,e*) lung, and (*c,f*) liver. DNA was isolated from a cigarette smoker and analyzed by ^{32}P-postlabeling analysis. The areas enclosed by dashes represent the diagonal radiation zone detected in smokers' heart and liver only by the nuclease P1 method. The circled areas indicate where nuclease P1-sensitive DNA adducts are detected only in the butanol method.

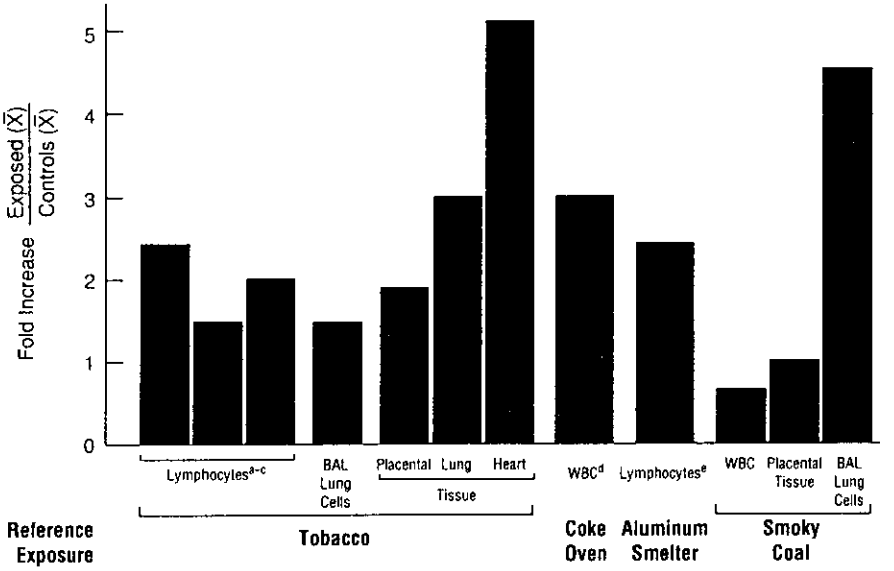

FIGURE 5. Effect of complex mixture exposure on the DNA adduct level of the exposed group divided by the control group. DNA was analyzed by the nuclease P1 version of the postlabeling assay. Three separate studies are reported for lymphocytes of smokers compared to nonsmokers; Savela and Hemminki (*35*),[a] Schoket et al. (*36*),[b] and the study reported here.[c] The data shown for coke oven white blood cell DNA are from Hemminki et al. (*34*)[d], and aluminum smelter lymphocyte DNA data are from Schoket et al. (*36*).[e]

centrations of PACs is due to the higher genotoxic potency of these emissions. Furthermore, the data presented in Figure 5 suggest that much higher levels of DNA adducts will be detected in target tissue (e.g., lung and heart) as compared to nontarget cells or tissue.

The three studies of DNA adducts in humans exposed to emissions from coke ovens, aluminum smelters, and smoky coal all demonstrate that the individual variation in DNA adduct levels in the exposed groups is much higher (e.g., ranging from 20- to 200-fold) than in the control groups (5- to 10-fold), as shown in Figure. 6. In each case, there are highly exposed individuals in which elevated DNA adduct levels are not detected and other individuals that have 1–2 orders of magnitude higher DNA adduct levels in both lung and blood cells compared to controls. These differences could be due to heterogeneity of exposure or differences in susceptibility factors (e.g., metabolism and DNA repair).

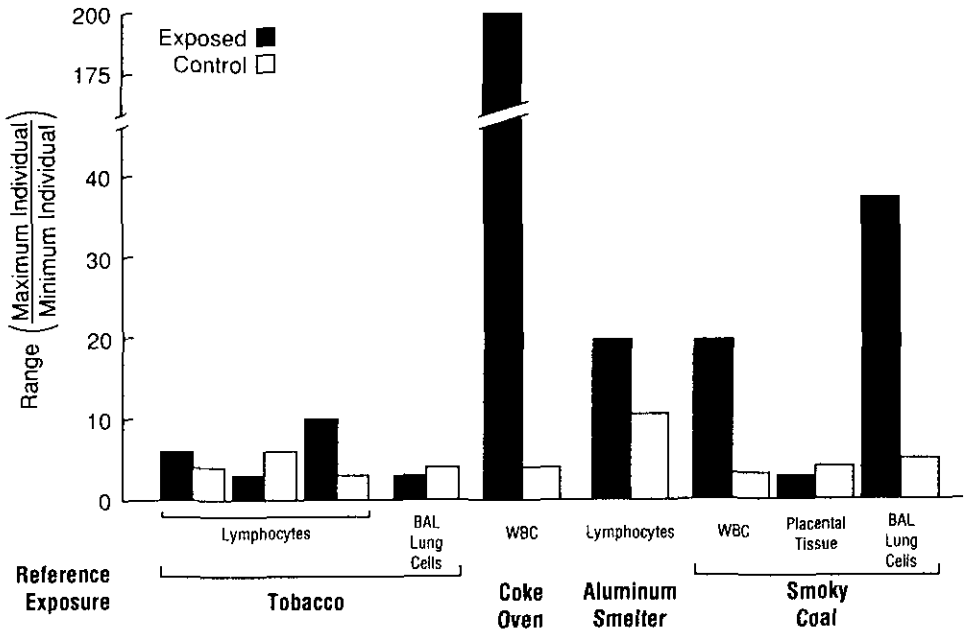

FIGURE 6. The range of interindividual variation for the human studies shown in Figure 5. The range was determined by dividing the maximum relative adduct level by the minimum relative adduct level value for the group.

Summary

In vitro and *in vivo* experimental models are useful for characterizing both the nature and level of DNA adducts resulting from complex mixture exposures. In particular, we have evidence that tobacco smoke exposures result in lower levels of detectable DNA adducts than other occupational and environmental PAC-containing mixtures. Furthermore, these studies suggest that although there are some similarities in the DNA adduct profiles between different PAC mixtures, there also appear to be differences in chromatographic properties, which may allow separation of DNA adducts from different sources of exposure.

Comparison of DNA adducts detected in cells and tissues of individuals exposed to tobacco smoke and coal-related emissions suggest that studies of target cells (e.g., lung and heart cells) will increase the sensitivity of these methods while providing DNA adduct dosimetry on target cell population. Individual variation in DNA adduct levels in both blood cells and lung cells of individuals exposed to high levels of these complex mixtures is an important factor in these human studies that needs to be better understood with respect to predicting risk.

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